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Segregation distortion in common wheat of a segment of *Thinopyrum intermedium* chromosome 7E carrying *Bdv3* and development of a *Bdv3* marker

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With 3 figures and 1 table

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Abstract

Yellow dwarf (YD) disease is one of the most destructive diseases of cereals worldwide. Wheat (*Triticum aestivum* L.)–*Thinopyrum intermedium* 7E(7D) substitution line P29 carries resistance to YD, known as *Bdv3*, that originates on the long arm of chromosome 7E of *Th. intermedium*, and the resistance was introgressed into wheat chromosome 7D as T7DS.7DL–7EL in the translocation lines P961341 and P98134. Until now, quantification of YD viruses in cereal crops was usually done by enzyme-linked immunosorbent assay (ELISA), which is time consuming and laborious. To facilitate this analysis, *SSR-Bdv3*, a diagnostic molecular marker, was developed in this study. The transmission of the *Th. intermedium* segment with *Bdv3* was investigated using the *SSR-Bdv3* marker and ELISA in F_2 and testcross progeny derived by crossing two wheat–*Th. intermedium* translocation lines to four common wheat cultivars. A *Th. intermedium* chromosome 7E segment in the translocation line P98134 was preferentially transmitted through male gametes in all of its crosses with the four wheat cultivars. However, the transmission frequency of the *Th. intermedium* 7E segment in another wheat–*Th. intermedium* translocation line, P961341, varied in different genetic backgrounds. The F_2 populations from reciprocal crosses of Chinese Spring and P961341 showed good fits to the expected ratio of 1 : 2 : 1. In this study, male preferential transmission for either chromosome 7E or chromosome 7D was observed in the progeny derived by crossing P961341 to other wheat cultivars.

Key words: *Triticum aestivum* — DNA marker — preferential transmission — barley yellow dwarf virus

Related wild species have been utilized extensively as sources of disease resistance alleles in the gene pool of common wheat (*Triticum aestivum* L.). At least 30 resistance genes have been transferred into wheat from the wheatgrasses *Thinopyrum intermedium* (2n = 6*x* = 42) and *Thinopyrum ponticum* (2n = 10*x* = 70) (Fedak et al. 2001). The gametic transmission frequencies of alien chromosomes are generally low. However, preferential transmission of alien chromosomes or chromosome segments in common wheat, termed 'gametocidal' (Endo 1978) or 'cuckoo' (Miller 1982) has been reported in various wild relatives such as *Aegilops sharonensis* (Miller 1982, King et al. 1991), *Ae. longissimum* (Mann 1975), *Ae. triuncialis* (Tsujimoto and Tsunewaki 1985), *Ae. caudata* (Endo and Katayama 1978), *Ae. cylindrica* (Endo 1979), *Ae. squarrosa* (Faris et al. 1998), *Secale cereale* (Lukaszewski et al. 1982), *Th. ponticum* (Kibirige-Sebunya and Knott 1983) and *Thinopyrum distichum* (Marais 1990).

Yellow dwarf (YD) caused by barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CYDV), and spread by several aphid vectors (primarily *Rhopalosiphum padi*, *Sitobion avenae*, *Rhopalosiphum madis* and *Schizaphis graminum*), is one of the most serious diseases of cereals worldwide (Comeau and Dubuc 1977, Gill 1980, Cisar et al. 1982, Liu et al. 2002). Little resistance to these viruses has been identified in wheat, but resistance has been introgressed from the wheatgrass *Th. intermedium* into wheat chromosome 7D as translocations (Ohm et al. 2005). To date, only one partially dominant resistance gene (*Bdv1*) has been identified in wheat (Singh et al. 1993). However, at least two genes, *Bdv2* (Larkin et al. 1995, Lin et al. 2006) and *Bdv3* (Ohm and Anderson 2007) each conditioning resistance to YD, have been identified in intermediate wheatgrass, *Th. intermedium* (host) Barkworth and Dewey [syn, *Agropyron intermedium* (host) P B], a hexaploid perennial Triticeae species (2n = 6*x* = 42, JJJ^sJ^sStSt) (previously $E_1E_1E_2E_2XX$, or $E^bE^bE^eE^eStSt$) (Sharma et al. 1995, Chen et al. 1998, Ayala et al. 2001). The disomic addition line L1 derived from TAF46, a partial wheat–*Th. intermedium* amphiploid (Cauderon and Rhind 1976), carries YD resistance on the long arm of the added group 7 chromosome 7St (Wang and Zhang 1996). Using L1 as the resistance source, a series of wheat–*Th. intermedium* translocation lines carrying YD resistance, designated *Bdv*2, were obtained by tissue culture (Banks et al. 1995) or by inducing homoeologous pairing using the *Ph1b* deletion (Xin et al. 1991, 2001).

The 7E (J or J^s genome)-(7D) substitution line P29 and 7E addition line P107 are wheat lines with YD resistance, designated *Bdv3*, similar to that of L1 but from a homoeologous *Th. intermedium* chromosome 7E (Sharma et al. 1995, Anderson et al. 1998, Crasta et al. 2000, Ohm et al. 2005). Their studies also demonstrated that *Bdv3* could be retained in backcross derivatives. Consequently, a series of translocation lines were developed from P29 and P107 by irradiation (Ohm et al. 2005). We, and other research groups, are currently introgressing this YD resistance into advanced breeding lines. The wheat–*Th. intermedium* translocation cultivar 'INW0316' developed at Purdue University, was released in 2003 (Ohm and Anderson 2007). Pollen sterility, especially under heat/cold and moisture stress, has been observed in these translocation lines, potentially indicating a transmission problem of a segment of *Th. intermedium* chromosome 7E.

Plants that express YD resistance can be identified by low virus titres (Cooper and Jones 1983) in enzyme-linked immunosorbent assays (ELISA). However, it is time consuming to maintain viruliferous aphids, and laborious to conduct large numbers of ELISA. Another attractive alternative to address the potential transmission problem induced by alien chromosome 7E is to use polymerase chain reaction (PCR)-based allele-specific (or alien segment-specific) markers to track the chromosome or chromosome segment. PCR-based dominant markers for *Bdv2* were reported (Stoutjesdijk et al. 2001,

Zhang et al. 2004). Unfortunately, some of these markers were not capable of detecting the 7E segment with *Bdv3*. Ayala et al. (2001) determined that microsatellite marker *Xgwm37* was closely linked to *Bdv2*, and we used it for selection of *Bdv3* (H. W. Ohm and J. M. Anderson, unpublished data). However, marker-assisted selection (MAS) with *Xgwm37* is not always feasible because the primers require highly purified DNA and the wheatgrass amplicons are most accurately observed by polyacrylamide electrophoresis. Adding bovine serum albumin (BSA) and polyvinylpyrrolidone (PVP) slightly increased the reliability of the *Xgwm37* primers, but the amplification continued to vary significantly between samples. Therefore, it was necessary to develop a more efficient PCR-based DNA marker to detect the transmission of the *Th. intermedium* chromosome 7E segment and to facilitate MAS for YD resistance in wheat breeding.

The objectives in this study were to: (i) investigate the transmission of the *Th. intermedium* 7E segment carrying *Bdv3* in different genetic backgrounds and whether transmission frequency is similar through male and female gametes and (ii) develop an improved diagnostic molecular marker for *Th. intermedium*-derived resistance (*Bdv2* and *Bdv3*) to facilitate this transmission study and MAS.

Materials and Methods

Plant material: The following plant materials were used in this study: (i) wheat–*Th. intermedium* 7E(7D) substitution line P29 carrying *Bdv3*, and wheat–*Th. intermedium* translocation lines, P961341 (Ohm et al. 2005) and P98134, which both carry *Bdv3*; (ii) wheat–*Th. intermedium* addition line L1 and wheat–*Th. intermedium* translocation cultivar 'Mackellar', which both carry *Bdv2*; (iii) hexaploid wheat lines/cultivars Chinese Spring (CS), VAN98W, B980696 and 'Foster' that do not contain *Bdv2* or *Bdv3*. Seeds of each line were planted in plastic 54 × 36 × 8 cm flats containing 10 rows of 20 seeds each; after emergence, seedlings were vernalized in a chamber with 12-h light/dark photoperiod regime for 65 days at 4°C, after which the seedlings were transplanted to a greenhouse.

CYDV resistance evaluation: *Bdv3* conditions resistance to both BYDV and CYDV. A previous study (Anderson et al. 1998) showed that resistance to CYDV results in very low ELISA optical density (OD) values allowing precise resistance or susceptible determinations. Therefore, in this study, YD resistance was evaluated by infestation with CYDV-viruliferous aphids and measuring CYDV titre using an ELISA as described by Anderson et al. (1998). Plants were grown in a growth chamber (18°C, 16-h photoperiod), and at the three-leaf stage the F_2 and testcross populations and the parental lines were infested with 10–20 CYDV-viruliferous *Rhopalosiphum padi* (L.) for 2 days and then sprayed with the insecticide malathion. Fourteen days postinfestation the tissue above the apical meristem was collected and the CYDV titre determined by ELISA. The susceptible CS and resistant parental lines (P961341 and P98134) controls had average OD of 1.06 and 0.06, respectively. Therefore, plants with an ELISA OD value >0.1 were considered to be susceptible to CYDV and missing *Bdv3*.

DNA isolation: Genomic DNA was isolated from seedling leaves using the method described by Saghai-Maroof et al. (1984) with minor modifications. The DNA extraction buffer contained 100 mM Tris–HCl buffer pH 8.0, 1.67% (w/v) hexadecyltrimethylammonium bromide (CTAB), 100 mM ethylenediaminetetraacetic acid disodium salt and 1.4 M NaCl. The concentration of each DNA sample was adjusted to 50 ng/μl after quantification on a Hoefer DyNA Quant 200 Fluorometer (Hoefer Pharmacia Biotech Inc., Dubuque, IA, USA) (Kong et al. 2005).

Development of the *SSR-Bdv3* DNA marker: PCR was carried out with genomic DNA from CS, wheat–*Th. intermedium* substitution line P29 carrying *Bdv3*, and 'Mackellar' carrying *Bdv2*. PCR amplifications were performed using 25-μl total volumes of 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP (Promega, Madison, WI, USA), 0.3 μM each of *Xgwm37* forward and reverse primers, 1 unit of *Taq* DNA polymerase (Promega), 50 ng of template DNA and 1.0 μg/μl BSA and 10 μg/μl PVP to improve the PCR amplification. The amplification profile consisted of one cycle at 94°C for 2 min, followed by 36 cycles of 30 s at 94°C, 45 s at 52°C and 1 min at 72°C, with a final extension of 7 min at 72°C. The PCR products were fractionated on 3.0% agarose gels using a mixture of 1 : 1 (w/w) MetaPhor and agarose in 0.5× TBE buffer (90 mM Tris–Borate, 1 mM EDTA, pH 8.0). Gels were stained with ethidium bromide and visualized with UV light. The wheat and *Thinopyrum*-specific genomic DNA fragments were cloned and sequenced as described previously (Kong et al. 2005). The only change was that the target PCR fragments were re-amplified under the same conditions using 1.0-μl aliquots of each excised fragment prior to cloning. Five clones for the fragments derived from CS, P29 and 'Mackellar' were sequenced by the Purdue University Genomics Centre DNA Sequencing Laboratory. Similarity searches were performed following a multiple sequence alignment produced by the software CLUSTAL X (Jeanmougin et al. 1998) (Fig. 1).

The oligonucleotide primers shown in Fig. 1 were designed from regions in P29, CS and 'Mackellar'-derived sequences having complete sequence identities using Primer3 (Rozen and Skaletsky 2000): forward primer: 5′-CTT AAC TTC ATT GTT GAT CTT A-3′ and reverse primer: 5′-CGA CGA ATT CCC AGC TAA ACT AGA CT-3′. The optimized PCR contained 50 ng template DNA, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.8 mM $MgCl_2$, 200 μM of each dNTP (Promega), 0.25 μM of each primer and 1 unit of *Taq* DNA polymerase (Promega). After initial heat denaturation at 94°C for 2 min, amplifications were performed for 35 cycles of a three-step PCR (30 s at 94°C, 45 s at 52°C and 1 min at 72°C) with a final extension for 7 min at 72°C. The amplified PCR products were fractionated on 3.0% agarose gels using a mixture of 1 : 1 (w/w) MetaPhor (Cambrex BioScience, Rockland, ME, USA) and agarose in 0.5× TBE buffer and photographed over a UV light source. These primers showed clear co-dominant polymor-

Fig. 1: Alignment of nucleotide sequences from P29 with *Bdv3* and non-*Bdv3* Chinese Spring (CS) (a), and nucleotide sequence comparison between P29 with *Bdv3* and 'Mackellar' with *Bdv2* (b). Three microsatellites (GA) were absent in 'Mackellar' (shown by dashes) compared with P29. Primer sequences are underlined

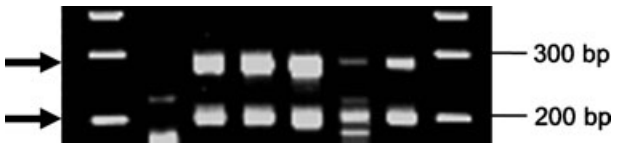

Fig. 2: The top and middle arrows show the fragments (288 bp and 206 bp, respectively) linked to the barley yellow dwarf virus (BYDV) resistance gene (*Bdv3*) from *Thinopyrum intermedium*. The bottom arrow shows the wheat band (164 bp) amplified in Chinese Spring (CS). M = 100-bp DNA ladder; 1 = CS; 2 = P961341 derived from P29, carrying *Bdv3*; 3 = P98134 derived from P29, carrying *Bdv3*; 4 = 'Mackellar' derived from L1, carrying *Bdv2*; 5 = L1, wheat–*Th. intermedium* addition line, carrying *Bdv2*; 6 = P29, wheat–*Th. intermedium* 7D/7E substitution line, carrying *Bdv3*

phisms between the YD-resistant line P29, the YD-susceptible line CS and the YD-resistant line 'Mackellar' (Fig. 2).

Data analysis: Data were analysed using the chi-square (χ^2) test to ascertain goodness of fit between the expected ratio for a single dominant gene and the observed phenotypic segregation. *SSR-Bdv3* developed in this study and other PCR-based markers including *Xgwm37* (Ayala et al. 2001) and *BYAgi* (Stoutjesdijk et al. 2001), previously found to be linked with *Th. intermedium*-derived *Bdv2,* were used to genotype the P961341/VAN98W//VAN98W BC_1F_1 population of 114 individuals.

Results

A diagnostic SSR marker for *Thinopyrum intermedium*-derived resistance to YD

DNA fragments, about 290 bp and 165 bp and specific to P29 and CS, respectively, were amplified by *Xgwm37* using an improved PCR protocol that contains PVP and BSA. Subsequently, the 290- and 165-bp fragments were cloned and sequenced. All five clones for the specific fragment from P29 had an identical 284-bp fragment, and the five clones for the specific band from CS had an identical 160-bp fragment as determined by a multiple alignment produced by the software CLUSTAL X. Oligonucleotide primers were designed based on this sequence alignment in an effort to develop a site-specific simple sequence repeat (SSR) marker (Fig. 1a). These primers identified two robust bands, 288 and 206 bp, in P29, and one robust band with 164 bp in the non-*Bdv3* CS (Fig. 2). The same primers were also used to screen the wheat–*Th. intermedium* addition line L1 and wheat–*Th. intermedium* translocation line 'Mackellar', which both carry *Bdv2*. The expected DNA fragments were amplified from both L1 and 'Mackellar' (Fig. 2). However, sequence alignment indicated that three (GA)-microsatellites for a total of six nucleotides were absent in 'Mackellar' compared with P29 (Fig. 1b). The *SSR-Bdv3* marker proved reliable not only with the wheat–*Th. intermedium* substitution and translocation lines and their derived segregating populations [e.g. F_2 (Fig. 3) and testcross BC_1F_1] but also with advanced breeding lines in our winter wheat breeding programme.

Transmission frequency of the *Thinopyrum intermedium* chromosome (7E) segment carrying *Bdv3*

The transmission ratios of the *Th. intermedium* chromosome 7E segment through male and female gametes in a number of genetic backgrounds, shown in Table 1, were analysed by chi-square analysis to test the goodness of fit between the expected and observed ratios scored with both the DNA marker and ELISA. *Thinopyrum intermedium*-specific DNA bands with 288 and 206 bp identified by DNA marker *SSR-Bdv3* were only present in wheat–*Th. intermedium* translocation lines, P98134 and P961341, but absent in all of the YD-susceptible parents, including CS, VAN98W, B980696 and 'Foster'. The wheat-specific DNA band (164 bp) was only present in the YD-susceptible wheat cultivars, but not in the translocation lines (Figs 2 and 3). The DNA marker results in all of the parent lines were verified by ELISA (data not shown).

When P98134 was crossed as the pollen parent with CS, the segregation ratio of *SSR-Bdv3* in the F_2 population was 75 : 56 : 10 (homozygous resistant : heterozygous resistant : homozygous susceptible), which was severely distorted from the expected ratio of 1 : 2 : 1 ($\chi^2 = 66.12$, $P < 0.01$, Table 1A). In conjunction with SSR-*Bdv3*, ELISA was carried out on 110 F_2 plants derived from CS/P98134; 99 showed resistance to YD, and only 11 were susceptible to YD, which indicated that the F_2 segregation ratio in the cross of CS/P98134 was strongly biased in favour of the translocation ($\chi^2 = 13.20$, $P < 0.01$, Table 1A). Similarly, the segregation ratio observed in the reciprocal cross of P98134/CS (55 homozygous resistant : 62 heterozygous resistant : eight homozygous susceptible) was also severely distorted from the expected ratio 1 : 2 : 1 ($\chi^2 = 35.35$, $P < 0.01$, Table 1A) based on the marker data. Thus, the origin of cytoplasm was probably not important in determining the segregation ratios in this cross. To decipher the distorted transmission frequencies of the wheat–*Th. intermedium* translocation, a testcross (F_1 from the translocation as female crossed with the YD-susceptible parent) and the reciprocal testcross (F_1 from the translocation crossed as male with the YD-susceptible parent) were made, and progeny were screened with the *SSR-Bdv3* marker. The transmission frequencies of the *Th. intermedium* segment by the female parent in the testcrosses of both CS/P98134//CS and P98134/CS//CS were normal or nearly normal ($\chi^2 = 0.008$, $P > 0.70$; $\chi^2 = 2.18$, $P > 0.10$, respectively). However, the male transmission frequencies of the 7E segment

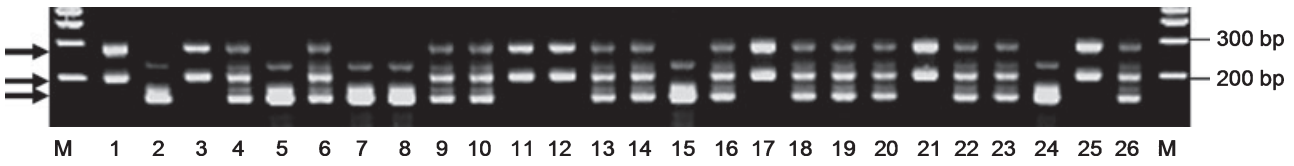

Fig. 3: *SSR-Bdv3* analysis in F_2 population of P961341/Chinese Spring (CS). The top and middle arrows show the fragments (288 bp and 206 bp, respectively) linked to the *Bdv3* resistance gene from *Thinopyrum intermedium*. The bottom arrow shows the band (164 bp) amplified in CS. M = 100 bp DNA ladder; 1 = P961341 carrying *Bdv3*; 2 = CS; 3–26 = F_2 population from P961341 × CS

Table 1: DNA marker and enzyme-linked immunosorbent assay (ELISA) data of F_2 and testcross obtained from the translocation lines (P98134 and P961341) and wheat lines and cultivars to test the transmission of the *Thinopyrum intermedium* chromosome 7E segment conferring *Bdv3*

			SSR-Bdv3				ELISA		
	Data set and cross	1 : 2 : 1 or 1 : 1	Number of plants tested	χ^2	P	3 : 1 or 1 : 1	Number of plants tested	χ^2	P
A	CS/P98134 F_2	75 : 56 : 10	141	66.12	< 0.01**	99 : 11	110	13.20	< 0.01**
	P98134/CS F_2	55 : 62 : 8	125	35.35	< 0.01**				
	CS/P98134//CS BC_1F_1	59 : 60	119	0.008	> 0.70				
	CS//CS/P98134 BC_1F_1	88 : 28	116	31.03	< 0.01**				
	P98134/CS//CS BC_1F_1	52 : 38	90	2.18	> 0.10				
	CS//P98134/CS BC_1F_1	93 : 23	116	42.24	< 0.01**				
B	P98134/VAN98W//VAN98W BC_1F_1	50 : 63	113	1.50	> 0.20	47 : 66	113	3.19	> 0.05
	VAN98W//P98134/VAN98W BC_1F_1	80 : 40	120	13.33	< 0.01**	75 : 45	120	7.50	< 0.01**
	B980696/P98134//B980696 BC_1F_1	52 : 58	110	0.33	> 0.50	50 : 60	110	0.91	> 0.30
	B980696//B980696/P98134 BC_1F_1	82 : 32	114	21.93	< 0.01**	82 : 32	114	21.93	< 0.01**
	P98134/'Foster'//'Foster' BC_1F_1	46 : 63	109	2.65	> 0.10				
	'Foster'//P98134/'Foster' BC_1F_1	73 : 37	110	11.78	< 0.01**				
C	CS/P961341 F_2	26 : 61 : 24	111	1.17	> 0.20	64 : 24	88	0.24	> 0.50
	P961341/CS F_2	28 : 68 : 30	126	0.86	> 0.30				
	CS/P961341//CS BC_1F_1	45 : 47	92	0.04	> 0.70				
	CS//CS/P961341 BC_1F_1	42 : 59	101	2.86	> 0.05				
	P961341/CS//CS BC_1F_1	44 : 34	78	1.28	> 0.20				
	CS//P961341/CS BC_1F_1	63 : 53	116	0.86	> 0.30				
D	B980696/P961341//B980696 BC_1F_1	41 : 50	91	0.89	> 0.30	38 : 53	91	2.47	> 0.1
	B980696//P961341/B980696 BC_1F_1	84 : 30	114	25.58	< 0.01**	84 : 30	114	25.58	< 0.01**
	'Foster'/P961341//'Foster' BC_1F_1	33 : 40	73	0.67	> 0.30				
	'Foster'//'Foster'/P961341 BC_1F_1	64 : 42	106	4.57	< 0.05*				
E	VAN98W/P961341 F_2	22 : 33 : 35	90	10.15	< 0.01**	57 : 38	95	11.40	< 0.01**
	P961341/VAN98W F_2	30 : 20 : 29	79	19.28	< 0.01**	64 : 39	103	9.09	< 0.01**
	P961341/VAN98W//VAN98W BC_1F_1	58 : 56	114	0.04	> 0.80	61 : 53	114	0.56	> 0.30
	VAN98W//P961341/VAN98W BC_1F_1	91 : 131	222	7.21	< 0.01**	39 : 60	99	4.45	< 0.05*

*Significantly different at P = 0.05, **Significantly different at P = 0.01.

in the reciprocal testcrosses of CS//CS/P98134 and CS//P98134/CS ($\chi^2 = 31.03$, $P < 0.01$ and $\chi^2 = 42.24$, $P < 0.01$, respectively) were significantly higher than expected, showing that the frequency of transmission of chromosome 7D was reduced from the expected 50% to 21.98% (two testcross data pooled). Therefore, the transmission ratios via pollen were strongly biased in favour of the 7E segment and, by inference, the YD resistance gene *Bdv3* (Table 1A).

Preferential transmission was also observed in other wheat cultivar backgrounds. For example, normal or nearly normal transmission of chromosome 7D through the female gametes was observed in all of the testcrosses; P98134/VAN98W//VAN98W ($\chi^2 = 1.50$, $P > 0.20$), B980696/P98134//B980696 ($\chi^2 = 0.33$, $P > 0.50$) and P98134/'Foster'//'Foster' ($\chi^2 = 2.65$, $P > 0.10$) (Table 1B). However, significantly lower male transmission rates (31.69%, three testcross data pooled) of the normal chromosome 7D were detected in all of the reciprocal testcrosses of VAN98W//P98134/VAN98W ($\chi^2 = 13.33$, $P < 0.01$), B980696//B980696/P98134 ($\chi^2 = 21.93$, $P < 0.01$) and 'Foster'//P98134/'Foster' ($\chi^2 = 11.78$, $P < 0.01$) (Table 1B). This preferential transmission in favour of the translocation chromosome via pollen was confirmed by ELISA (Table 1B) in the reciprocal testcrosses of both VAN98W//P98134/VAN98W ($\chi^2 = 7.50$, $P < 0.01$) and B980696//B980696/P98134 ($\chi^2 = 21.93$, $P < 0.01$). The female gametic transmission frequencies were close to expected in the testcrosses of both P98134/VAN98W//VAN98W ($\chi^2 = 3.19$, $P > 0.05$) and B980696/P98134//B980696 ($\chi^2 = 0.91$, $P > 0.30$) based on ELISA data (Table 1B).

For the purpose of confirming the male and female transmission frequencies of the *Th. intermedium* segment conferring *Bdv3*, another wheat–*Th. intermedium* translocation line, P961341, which has a shorter alien segment than the approximate 2/3 of the long arm of 7E in P98134 (J. M. Anderson, unpublished data) was also investigated. F_2 populations of CS/P961341 and P961341/CS segregated, respectively, 26 homozygous resistant : 61 heterozygous resistant : 24 homozygous susceptible (Fig. 3) and 28 : 68 : 30. These numbers showed good fits to the expected 1 : 2 : 1 ratio ($\chi^2 = 1.17$, $P > 0.20$ and $\chi^2 = 0.86$, $P > 0.30$, respectively) (Table 1C). Similar to the cross of CS and P98134, the origin of the cytoplasm was probably not important in determination of the segregation ratios in this cross. Unlike P98134, among 88 F_2 plants of CS/P961341 examined by ELISA, 64 plants were resistant and 24 plants were susceptible to YD, consistent with a normal 3 : 1 segregation ($\chi^2 = 0.24$, $P > 0.50$, Table 1C). Subsequent testcrosses verified that the transmission ratios through either male or female gametes were normal or nearly normal ($0.04 \leq \chi^2 \leq 2.86$, $P > 0.05$–0.70, Table 1C).

However, the transmission ratios resulting from testcrosses of P961341 with wheat line B980696 and cultivar 'Foster' did reveal a range in the severity of gametocidal effects in male gametes ($\chi^2 = 25.58$, $P < 0.01$ and $\chi^2 = 4.57$, $P < 0.05$, respectively) (Table 1D). The female gametic transmissions of the 7E segment observed in testcrosses B980696/P961341//B980696 and 'Foster'/P961341//'Foster' ($\chi^2 = 0.89$, $P > 0.30$ and $\chi^2 = 0.67$, $P > 0.30$, respectively) (Table 1D) were basically normal. In addition, based on ELISA test (Table 1D), the female transmission of 7D was close to normal in the testcross B980696/P961341//B980696 ($\chi^2 = 2.47$, $P > 0.10$). Whereas the transmission of 7D through male gametes was severely reduced to favour 7E in the reciprocal testcross B980696//P961341/B980696 ($\chi^2 = 25.58$, $P < 0.01$).

The segregation ratios determined in the F_2 population of VAN98W/P961341 (22 homozygous resistant : 33

heterozygous resistant : 35 homozygous susceptible) and the reciprocal F_2 of P961341/VAN98W (30 homozygous resistant : 20 heterozygous resistant : 29 homozygous susceptible) were both strongly distorted from the expected ratio of 1 : 2 : 1 for a single dominant gene ($\chi^2 = 10.15$, $P < 0.01$; $\chi^2 = 19.28$, $P < 0.01$, respectively) (Table 1E). The ELISA test (Table 1E) was in agreement with the above PCR marker data in F_2 of VAN98W/P961341 (57 YD-resistant : 38 YD-susceptible, $\chi^2 = 11.40$, $P < 0.01$) and the reciprocal F_2 of P961341/VAN98W (64 YD-resistant : 39 YD-susceptible, $\chi^2 = 9.09$, $P < 0.01$). The transmission frequencies determined by the testcross P961341/VAN98W//VAN98W ($\chi^2 = 0.04$, $P > 0.80$) and the reciprocal testcross VAN98W//P961341/VAN98W ($\chi^2 = 7.21$, $P < 0.01$) revealed a male preferential transfer of wheat chromosome 7D instead of the gametocidal effect observed in the translocation line P98134 above. Further, the ELISA test confirmed that the male transmission frequency was significantly distorted in favour of wheat chromosome 7D in the reciprocal testcross of VAN98W//P961341/VAN98W ($\chi^2 = 4.45$, $P < 0.05$), while the transmission ratio through female gametes was normal in the testcross of P961341/VAN98W//VAN98W ($\chi^2 = 0.56$, $P > 0.30$) (Table 1E).

Discussion

Yellow dwarf viruses are a group of poleroviruses and luteoviruses that are obligately transmitted by aphids and are the most economically important viral pathogens of cereal grains worldwide (Cisar et al. 1982, McKirdy and Jones 1996, Liu et al. 2002). Study of YD is complex, as it involves interactions among a vector, a plant and a virus, and symptom expression is highly dependent on environmental conditions, virus serotypes, plant genetic backgrounds and plant growth stages at inoculation (Ayala et al. 2002). Determining if the transmission frequency of *Thinopyrum* chromosome 7E was the same through male and female gametes required the analysis of 2 896 plants in segregating progeny. This analysis was enhanced by the development of *SSR-Bdv3* as it allowed the plants to be readily genotyped. This marker facilitates large scale screening of *Bdv2* and *Bdv3* by providing tools for high-throughput marker evaluations which do not require the presence of the virus and is much more convenient than ELISA (Fig. 2). The six nucleotide difference observed between P29 and 'Mackellar'-derived *Thinopyrum*-specific amplicons may be sufficient for reliable detection on capillary electrophoresis, but probably not for analysis by high resolution agarose gel electrophoresis. However, this difference may be quite useful when attempting to combine *Bdv2* and *Bdv3* into the same wheat line. The PCR-based DNA marker data was validated by the ELISA analysis of 12 of the F_2 and testcross segregating populations.

Segregation distortion can be defined as deviations from normal Mendelian segregation, and has been recognized as a common genetic phenomenon in many organisms. Common wheat ($2n = 6x = 42$) belongs to the tribe Triticeae, which also contains numerous related wild species to which it can be sexually hybridized. Introgressed Triticeae chromosomes that are preferentially transferred into common wheat were termed 'gametocidal' (*Gc*-genes) (Endo 1978) or 'cuckoo' (Miller 1982). A previous study on $4S^l$ from *Ae. sharonensis* showed that the chromosome $4S^l$ was transmitted at a very high frequency (at least 97.8%) through either male or female gametes in all crosses, and the genetic background had a small effect on transmission (King et al. 1991). However, different results were reported from a study of chromosome 3C of *Ae. cylindrica*, which was transmitted at a higher frequency only through female gametes in some genetic backgrounds (Endo 1979). Complete transmission of a *Triticum durum* chromosome via the female parent was demonstrated by the analysis of BC_1 progeny obtained from the F_1 of *T. durum*/*Triticum persicum* (Manabe et al. 1999). *Thinopyrum* (also called *Lophopyrum* or *Agropyron*) has been widely exploited for wheat improvement by crossing with *Triticum* species. Studies on *Th. ponticum* chromosome $7el_2$ showed that *Thinopyrum* $7el_2$ had gametocidal effects that resulted in high transmission ratios through female gametes (Kibirige-Sebunya and Knott 1983). Studies on translocations involving another *Th. ponticum* chromosome, $7el_1$, carrying *Lr19* indicated that the *Thinopyrum* chromosome $7el_1$ was transmitted normally in Thatcher and CS backgrounds and had no apparent deleterious effects on the gametes carrying it (Sharma and Knott 1966, McIntosh et al. 1976). However, when *Lr19* was transferred to other genetic backgrounds very high levels of distortion were observed (McIntosh et al. 1995). Also, meiotic self-elimination was not observed in a 7EL/7DL line in which the 7E segment was shortened (Zhang et al. 2005).

In the present study, the transmission of the *Th. intermedium* chromosome 7E segment carrying *Bdv3* was investigated using the *SSR-Bdv3* marker and ELISA in F_2 and testcross progeny derived by crossing two wheat–*Th. intermedium* translocation lines to four common wheat cultivars. Significant deviations from expected Mendelian segregation were detected in the F_2 of P98134 and CS (Table 1A). The preferential transmission of the *Th. intermedium* 7E segment through male gametes was verified by testcrosses. Of the 576 testcross individuals obtained from the F_1s used as male, 416 (72.22%) ($\chi^2 = 113.78$, $P < 0.01$, pooled data from five testcross) (Table 1A and B) had the 7E segment as shown by the presence of the *SSR-Bdv3*. Transmission of a 7E segment in another wheat–*Th. intermedium* translocation line, P961341, was also affected by the genetic background. The F_2 populations from the cross of CS and P961341 showed a normal 1 : 2 : 1 segregation for a single dominant gene ($\chi^2 = 1.86$, $P > 0.10$, both reciprocal data pooled) (Table 1C). However, preferential male transmissions for either *Th. intermedium* chromosome 7E or wheat chromosome 7D were observed in different crosses of P961341 with different common wheat cultivars (Table 1D and E), which may be indicative of a strong gametocidal response, or may be the result of stronger expression of gametocidal effects in the gametes. Because *Bdv3* does exhibit significant segregation distortion, the linked marker *SSR-Bdv3* will be very useful in correctly selecting lines carrying *Bdv3,* irrespective of the direction the cross is made. Further study is needed to determine if the differences in transmission of the *Th. intermedium* chromosome segments in P98134 and P961341 are affected by translocation length, different genetic backgrounds or both factors.

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